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Development of an AAV-2 Vector for the Treatment of Hemophilia B

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Hemophilia B, the deficiency in the clotting factor IX (FIX), is an X-linked recessive disorder that occurs in about one in 25,000 males in the U.S. Although the use of plasma-derived or recombinant FIX has greatly extended the life span of these patients, they still remain susceptible to a number of pathologies of the disease including retinopathies, joint bleeding leading to joint dysfunction, vascular occlusive disease, and internal bleeding events that can be life threatening. The use of replication deficient AAV vectors to deliver the human FIX gene offers the opportunity to achieve stable therapeutic levels of FIX in hemophilia patients and the potential to avoid many of these pathologies. We have previously demonstrated the stable expression of human factor IX (hFIX) protein in mice and canine factor IX (cFIX) protein in hemophilic B dogs following gene transfer with a rAAV encoding vector, however, we wished to further optimize the vector transgene cassette used in these studies for tissue specificity and strength of expression. First, several liver-specific promoters were explored and tested. Our rationale was that liver-specific FIX expression would limit gene expression to cells that normally express Factor IX and would help reduce the potential of inducing an anti-hFIX immune response. In addition, the construct was further optimized for expression by the inclusion of additional regulatory elements. Vectors were tested both in cell culture and in vivo for their effect on transgene expression, in comparison to previous generations of AAV-hFIX construct. Results demonstrated that constructs which contained both the b-globin intron and the woodchuck post-transcriptional regulatory element (WPRE) in combination with a liver specific promoter resulted in the strongest expression in vivo, with serum hFIX levels being 5 fold greater than that achieved for an MFG promoter construct. These levels remained stable for over 78 weeks. Although portal vein administration of vector appears to be an effective route of administration to achieve AAV transduction of the liver, it is rather invasive. We therefore also examined alternative delivery routes that maybe more appropriate in the context of hemophilia such as intra-venous (I.V.) and direct liver administration. Results indicate that while the efficiency of gene transfer with I.V. administration of this vector is slightly lower than portal vein administration (70-80% of portal vein values), this route of delivery achieves therapeutic levels of circulating hFIX. Biodistribution was determined by analyses of tissue for the presence of viral vector using real time PCR and demonstrated the predominant organ of transduction was the liver, regardless of administration route. Further studies utilizing this vector will also be presented.

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